

AD _____

Award Number: DAMD17-99-1-9456

TITLE: Structure of the Tetrameric p53 Tumor Suppressor Bound to
DNA

PRINCIPAL INVESTIGATOR: Ronen Marmorstein, Ph.D.

CONTRACTING ORGANIZATION: The Wistar Institute
Philadelphia, Pennsylvania 19104

REPORT DATE: May 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE May 2000	3. REPORT TYPE AND DATES COVERED Annual (15 Apr 99 - 14 Apr 00)	
4. TITLE AND SUBTITLE Structure of the Tetrameric p53 Tumor Suppressor Bound to DNA			5. FUNDING NUMBERS DAMD17-99-1-9456	
6. AUTHOR(S) Ronen Marmorstein, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Wistar Institute Philadelphia, Pennsylvania 19104 E-MAIL: marmor@wistar.upenn.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) The p53 transcriptional activator binds to DNA as a tetramer to activate the transcription of genes involved in cell cycle arrest and apoptosis, and alterations in the DNA-binding domain of p53 are the most common genetic changes found to date in breast cancer. The overall goal of the proposal is to determine the X-ray crystal structure of tetrameric forms of p53 bound to DNA. Over the last year we have made significant progress towards achieving this goal. Specifically, we have successfully cloned, overexpressed and purified to homogeneity two relevant protein constructs of p53 that are competent for tetramer formation on DNA; p53(98-292), and p53(86-351). We are pursuing the structure determination of both of these protein constructs bound to DNA in parallel. For the p53(98-292) protein construct we have obtained crystals of a p53/DNA complex and a structure determination is in progress, and for the p53(86-351) construct cocrystallization trials with DNA are in progress. The structure of these p53/DNA complexes will provide a mechanistic understanding into the structural basis underlying p53 mutations, and will provide a framework for the structure-based design of drugs that will be useful in the treatment of p53-mediated breast cancer.				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 9	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.


PI - Signature

5/9/00
Date

Table of Contents

Cover.....	
SF 298.....	
Foreword.....	
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6
Appendices.....	8-9

(5) INTRODUCTION

Alterations of the p53 tumor suppressor gene are the most common genetic changes found to date in breast cancer, suggesting that the gene plays a central role in the development of the disease (Hollstein et al., 1994; Lee and Bernstein, 1995; Ozbun and Butel, 1995). p53 activity is mediated by a tetrameric form of the protein that binds to DNA to activate the transcription of genes involved in cell cycle arrest or apoptosis (Donehower and Bradley, 1993; Lee and Bernstein, 1995). The p53 protein contains four functionally distinct domains: an N-terminal transactivation domain, a central core DNA binding domain, a tetramerization domain, and a C-terminal regulatory domain (Pavletich et al., 1993; Wang et al., 1993). The vast majority of tumor-derived p53 mutations are localized to the core domain and thus prevent p53 from binding DNA (Hollstein et al., 1991). The overall goal of the proposal is to determine the X-ray crystal structure of a tetrameric form of p53 bound to DNA. The specific technical objectives of our proposal are to (1) Prepare p53 protein and DNA target sites for p53/DNA cocrystallization (tasks 1-4), (2) Crystallize the p53/DNA complex for structure determination using X-ray crystallography (tasks 5 and 6), (3) Determine the three-dimensional structure of a p53/DNA complex (tasks 7-13), and (4) Overexpress and purify to homogeneity the p53-T284R mutant protein that is able to rescue common tumor-derived p53-mutations, crystallize it bound to DNA, and determine the structure of the p53-T284R/DNA complex (tasks 14-20). The structure of the p53/DNA complex will provide a mechanistic understanding into the structural basis for how p53 mutations result in mammary carcinomas, and a comparison with the p53-T284R/DNA structure will provide a framework for the structure-based design of drugs that may mimic the T284R mutation (Wieczorek et al., 1996) and thus will be useful in the treatment of p53-mediated breast cancer.

(6) BODY

Over the last year we have made significant progress towards achieving the overall goal of the proposal. In our initial attempts to identify p53 molecules that were amenable to preparation for structural analysis, we screened p53 molecules from different species and have discovered that p53 from mouse was the most amenable to biochemical and subsequent structural analysis. Specifically, we have successfully cloned, overexpressed and purified to homogeneity two relevant protein constructs of p53 that are competent for tetramer formation on DNA; p53(98-292) harboring the p53 core domain, and p53(86-351) harboring the core-linker-tetramerization region of p53 (task 1) (Figs. 1 and 2). We are pursuing the structure determination of both of these protein constructs bound to DNA in parallel. For the p53(98-292) protein construct we have progressed to task 7 of technical objective 3, and for the p53(86-351) construct we have progressed to task 5 of technical objective 2.

A more detailed analysis of our accomplishments follows. We have used gel-shift analysis to show that both p53(98-292) and p53 (86-351) protein constructs are competent to bind an idealized p53 DNA target site as a tetramer (task 3), and have also purified each of the 10 p53 DNA target sites outlined in our proposal for cocrystallization trials (task 2). A subset of the complexes showed monodisperse behavior when analyzed using dynamic light scattering and by gel filtration (task 4) and these were set up for crystallization trials (task 5). We have not yet obtained protein/DNA complex crystals with the p53 (86-351) protein construct. For the p53(98-292) protein constructs, we have obtained crystals under several different crystallization conditions, and the crystals that diffract to the highest resolution when analyzed using a home X-ray source grow from a solution containing 0.2 mM p53/20-bp DNA, 8% PEG4000, 20 mM Mes, pH 5.6, 200 mM KCl and 50 mM MgCl₂ (task 6). We have collected a preliminary 3.0 Å resolution data set from these crystals (Table 1) and analysis of the data indicates the crystals form in the space group C222₁ with unit cell dimensions of a=75.4 Å, b=121.3 Å, and c=185.5 Å (task 7) (Fig. 3). Consideration of the unit cell volume of these crystals suggests that they contain the p53 protein bound as a tetramer to one DNA duplex with about 50% solvent content in the asymmetric unit cell.

We are currently initiating the structure determination of the tetrameric p53(98-292)/DNA complex with the Molecular Replacement technique using the monomeric p53/DNA complex (Cho et al., 1994) as a search model. In parallel, we are preparing heavy atom derivatives of p53(98-292), including a seleno-methionine derivatized p53 protein, to obtain model-unbiased phases for the p53(98-292)/DNA structure. We are also arranging a synchrotron trip so that we can obtain higher resolution data (better than 3.0 Å) for the tetrameric p53(98-292)/DNA complex. As mentioned earlier, crystallization trials of the longer p53(86-351) construct bound as a tetramer to DNA are also underway.

(7) KEY RESEARCH ACCOMPLISHMENTS

- ☐ Overexpression and purification to homogeneity of a p53 core domain construct, p53(98-292).
- ☐ Overexpression and purification to homogeneity of a p53 (86-351) protein construct harboring the core domain, linker region and tetramerization domain.
- ☐ Crystallization of a tetramer of p53(98-292) bound to a 20-bp DNA duplex.
- ☐ Data collection and characterization of crystals of the p53(98-292)/20-bp DNA complex.

(8) REPORTABLE OUTCOMES

No reportable outcomes to date.

(9) CONCLUSIONS

During the first year of the funding period we have made considerable progress towards achieving our proposed goals. Specifically, we have overexpressed and purified to homogeneity the p53(86-351) protein and cocrystallization efforts with DNA are underway (through task 5 of technical objective 2). In addition, we have obtained crystals and collected data from crystals of a p53(98-292) tetramer bound to DNA (through task 7 of technical objective 7).

By the end of the second year of the grant period we expect to be well on our way to completion of the structure determination of both the p53(98-292) and p53(86-351) protein constructs bound as tetramers to DNA (technical objective 3, tasks 9-13). These structures will set the stage for the completion of the final technical objective of the proposal (technical objective 4) during the final year of the proposal. The final objective of the proposal is to determine the structure of the DNA complex with the T284R p53 mutant protein that rescues tumor derived mutations. A comparison of this structure with the native structures is expected to provide a framework for the structure-based design of drugs that may mimic the T284R mutation and thus will be useful in the treatment of p53-mediated breast cancer.

(10) REFERENCES

- Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994). Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* 265, 346-355.
- Donehower, L. A., and Bradley, A. (1993). The tumor suppressor p53. *Biochim. Biophys. Acta* 1155, 181-205.
- Hollstein, M., Rice, K., Greenblatt, M. S., Soussi, T., Fucks, R., Sorlie, T., Hovig, E., Smith-sorensen, B., Montesano, R., and Harris, C. C. (1994). Database of p53 gene somatic mutations in human tumors and cell lines. *Nucleic Acids Res.* 22, 3551-3555.

Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991). p53 mutation in human cancers. *Science* 253, 49-53.

Lee, J. M., and Bernstein, A. (1995). Apoptosis, cancer and the p53 tumor suppressor gene. *Cancer Metastasis Rev.* 14, 149-161.

Ozbun, M. A., and Butel, J. S. (1995). Tumor suppressor p53 mutations and breast cancer: a critical analysis. *Adv. Cancer Res.* 66, 71-141.

Pavletich, N. P., Chambers, K. A., and Pabo, C. O. (1993). The DNA binding domain of p53 contains the four conserved regions and the four major mutation hot spots. *Genes & Develop.* 7, 2556-2564.

Wang, Y., Reed, M., Wang, P., Stenger, J. E., Mayr, G., Anderson, M. E., Swhwedes, J. F., and Tegtmeyer, P. (1993). p53 domains: identification and characterization of two autonomous DNA-binding domains. *Genes & Dev.* 7, 2575-2586.

Wieczorek, A. M., Waterman, J. L. F., Waterman, M. J. F., and Halazonetis, T. D. (1996). Structure-based rescue of common tumor-derived p53 mutants. *Nature Medicine* 2, 1143-1146.

(11) APPENDICES

Figures 1, 2 and 3; and Table 1.

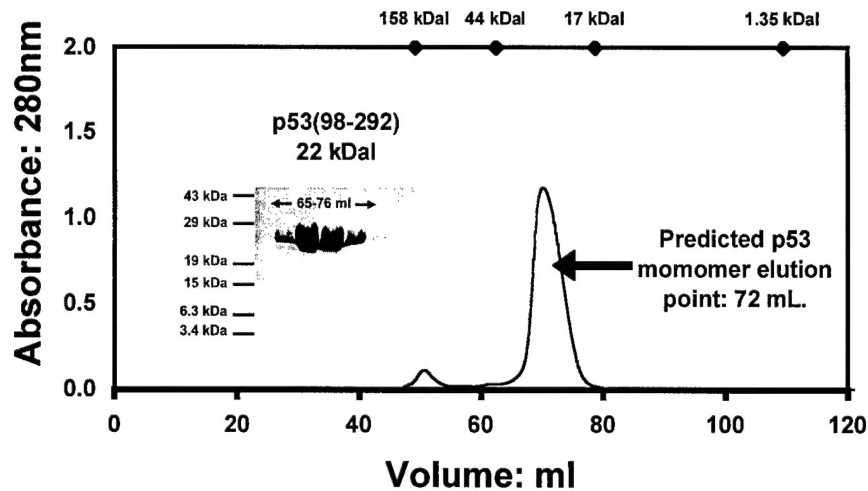


Figure 1. Purification of the p53(98-292) core domain. p53(98-292) was purified using a combination of cation exchange and gel filtration chromatography. The final gel filtration chromatograph on a Superdex-75 gel-filtration FPLC column is shown with the corresponding peak fractions illustrated on the embedded SDS-PAGE gel.

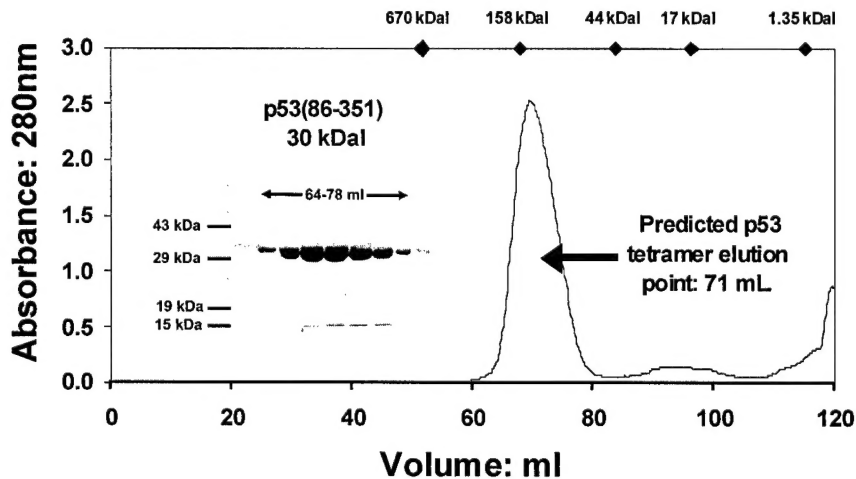


Figure 2. Purification of the p53(86-351) core-linker-tetramerization domain construct. p53(86-351) was purified using a combination of cation exchange and gel filtration chromatography. The final gel filtration chromatograph on a Superdex-200 gel-filtration FPLC column is shown with the corresponding peak fractions illustrated on the embedded SDS-PAGE gel.

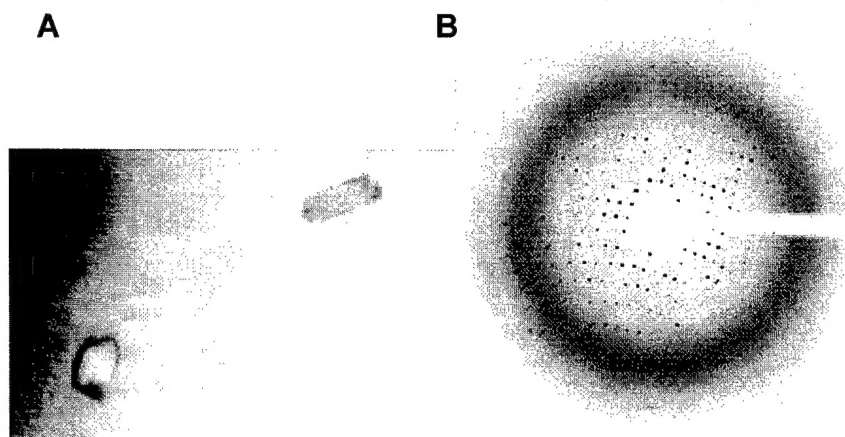


Figure 3. Crystals and diffraction from a tetrameric p53(98-292)/20-bp DNA complex. (A) Crystals of the complex are shown with the largest dimension of 0.5 mm. (B) Diffraction pattern from the crystals shown in (A). The highest resolution reflection occurs at 3.0 Å.

Table 1. Summary of crystallographic parameters for crystals of the tetrameric p53(98-292)/20-bp DNA complex.

Parameter	<i>C2 22₁</i>
Cell parameters	<i>a</i> =75.38Å <i>b</i> =121.30Å <i>c</i> =185.45Å
no. of observations	70239
no. of unique reflections	31964
Tetramer per Asy. Unit	1
<i>V_m</i> (Å ³)	2.12
resolution (Å)	3.0
overall completeness (last shell)	97.2 (95.3) %
Overall <i>I</i> /sigma (last shell)	15.4 (2.3) %
<i>R</i> merge (last shell)	7.10 (32.3) %